U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2015/2016

Tito Xavier da Costa

Acute metformin intoxication: diagnosis, management and prognosis

março, 2016

Tito Xavier da Costa

Acute metformin intoxication: diagnosis, management and prognosis

Mestrado Integrado em Medicina

Área: Nefrologia

Tipologia:Monografia

Trabalho efetuado sob a Orientação de:

Doutora Carla Alexandra dos Santos Araújo

Trabalho organizado de acordo com as normas da revista:

Revista Portuguesa de Nefrologia e Hipertensão

março, 2016

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR
PROJETO DE OPÇÃO
E DISSERTAÇÃO MONOGRAFIA

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Tito Xavier da Costa, abaixo assinado, nº mecanográfico 200908210, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 1/04/2016

Assinatura conforme cartão de identificação:

Tito Xavier da Costa

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR
PROJETO DE OPÇÃO
DISSERTAÇÃO / MONOGRAFIA

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Tito Xavier da Costa

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
200908210	

DESIGNAÇÃO DA ÁREA DO PROJECTO

Nefrologia

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Acute metformin intoxication: diagnosis, management and prognosis

ORIENTADOR

Doutora Carla Alexandra dos Santos Araújo

COORIENTADOR (se aplicável)

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☒
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☐

Faculdade de Medicina da Universidade do Porto, 1/04/2016

Assinatura conforme cartão de identificação: Tito Xavier da Costa

Aos meus avós

Acute metformin intoxication: diagnosis, management and prognosis

Intoxicação aguda por metformina: diagnóstico, gestão e prognóstico

Authors: Tito X. Costa[2], Carla S. Araújo[1,2]

1- Nephrology Department, São João Hospital Centre, Porto, Portugal

2- Faculty of Medicine, University of Porto, Portugal

Corresponding author

Tito Xavier da Costa

Morada: Rua da Quinta Seca, 214, 3ºesq, 4460-393 Matosinhos, Porto, Portugal

Telfone: 933098059

E-mail: tito.fmup16@gmail.co

Abstract

Background: Metformin intoxication can potentially originate a lactic acidosis of variable severity, but possibly fatal, diagnosis and prompt treatment of which are crucial. The difficulty in establishing a clear cause and effect relationship arises from the multitude of biologic actions exerted by metformin.

Objective: Review of literature in order to compile the current knowledge regarding the diagnosis, management and prognosis of metformin associated lactic acidosis.

Methods: Data were collected using PubMed database researching the terms "metformin" and "lactic acidosis". After selection of 29 titles and abstracts, 25 full-text articles were consulted.

Results: The direct mechanism through which metformin induces lactic acidosis remains incompletely understood. Its anti-gluconeogenic actions and respiratory chain inhibition appear to play a key role. Prognosis depends mainly on the presence of comorbid conditions, rather than the degree of either metformin or lactate accumulation. And independent variable that seems to influence prognosis is prothrombin time at admission. Prolonged dialysis appears to be an adequate treatment.

Conclusions: Prompt recognition and institution of both supportive treatment and renal replacement techniques are appropriate regarding the management of metformin associated lactic acidosis, being the most relevant aspects in improving the outcome of this condition.

Keywords: metformin, lactic acidosis

Resumo

Introdução: A intoxicação por metformina tem o potencial de originar uma acidose láctica de gravidade variável, mas potencialmente fatal, cujo expedito reconhecimento e instituição do tratamento são fulcrais. A dificuldade de determinação de uma relação

direta de causa-efeito advém da miríade de ações biológicas exercidas pela metformina, esclarecimento das quais continua em expansão.

Objetivo: Revisão da literatura com o objectivo de compilar o conhecimento atual relativo ao reconhecimento, tratamento e prognóstico da acidose láctica associada à metformina.

Métodos: Pesquisa bibliográfica na base de dados científica PubMed utilizando os termos "metformina" e "acidose láctica", com seleção inicial de 29 artigos, e utilização de 25 destes na realização do presente trabalho.

Resultados: O mecanismo direto através do qual a metformina induz acidose láctica e o modo como isto se traduz no quadro clínico continuam por elucidar totalmente. As ações anti-gliconeogénicas bem como a inibição da cadeia respiratória parecem desempenhar um papel preponderante. O prognóstico parece amplamente dependente da presença de comorbilidades e não do grau de acumulação de metformina ou lactato. Uma variável independente que aparenta valor prognóstico é o tempo de protrombina à admissão. O tratamento com diálise de longa duração mostra-se adequado.

Conclusões: O rápido reconhecimento desta entidade e pronta instituição de tratamento de suporte e técnicas de substituição renal afiguram-se adequados na gestão da intoxicação aguda por metformina, sendo os aspectos mais relevantes na melhoria do prognóstico.

Palavras-chave: metformina, acidose láctica.

Introduction

Metformin has a well-established role as the mainstay of type 2 *diabetes mellitus* pharmacologic therapy, either in alone or in combination with other agents, due to its extensive track record of safety and tolerability, low cost, and efficacy[1]. Despite the favourable safety profile, metformin therapy may be associated with the development of lactic acidosis, a scenario that must be kept in mind considering the amount of patients potentially at risk- i.e. type 2 diabetics under metformin therapy.

With an estimated incidence ranging from 3 to 47 cases per 100.000 patient-years[2,3,4], this clinical entity carries an elevated mortality rate, up to 50% in some series[2]. Metformin intoxication by overdose, either accidental or intentional, is uncommon. The majority of cases occur when a precipitating event or predisposing condition, while under therapeutic metformin levels, impair the renal clearance of the drug[2].

The mechanism underlying metformin-induced lactic acidosis remains incompletely understood, perhaps as a consequence of the difficulty, over the years, of clearly establishing metformin's exact mode of action. Importantly, recent investigations have carried out significant advances in pinpointing specific molecular targets, focusing on how metformin exerts its therapeutic actions. However, the relationship between the abnormally elevated levels of metformin and the development of lactic acidosis is neither straightforward nor linear, and a plenitude of coexisting conditions usually confounds the clinical picture.

Metformin intoxication presentation is unspecific, frequently affected by eventual coexisting pathologies, and most commonly characterized by vomiting and diarrhoea[3]; with hypothermia and respiratory failure also being reported[5], and eventually progressing to severe lactic acidosis. There may be, however, asymptomatic presentations, which contributes to the difficulty of early recognition of the clinical picture and expeditious institution of therapeutic manoeuvres[6].

After the diagnosis is established with fair certainty, haemodialysis appears to constitute the most appropriate course of action regarding treatment of acute metformin intoxication, and its early institution seems advisable[7], with prolonged haemodialysis being the method of choice[2;8].

The purpose of this review is to compile the current understanding regarding metformin intoxication, its recognition and management, and also the possible

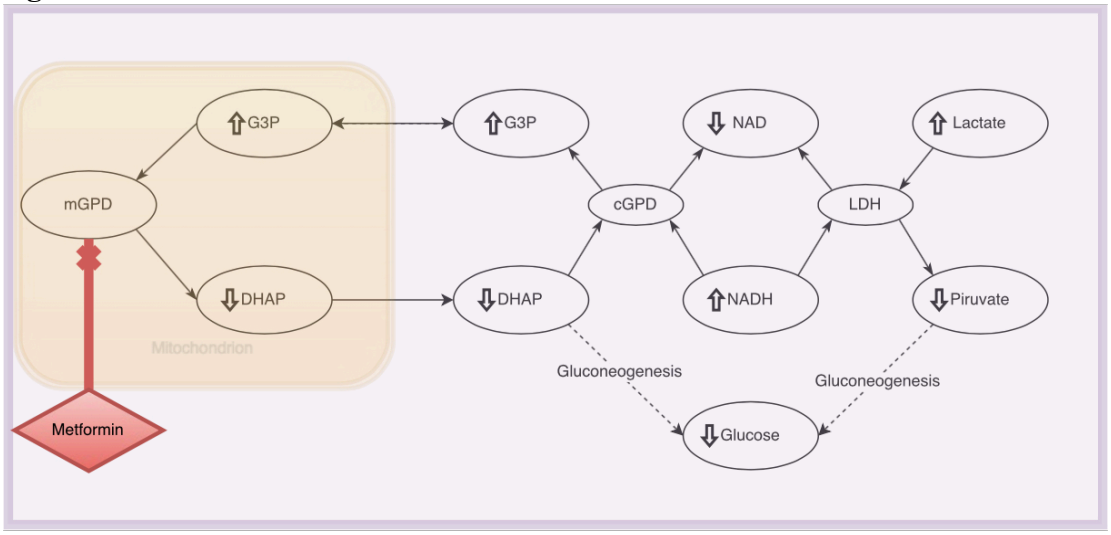
prognostic indicators amenable to give some insight into individual outcomes of this unusual, but conceivably fatal condition.

Metformin- Mode of action and induction of lactic acidosis

Despite having been in clinical use for over 60 years, the full spectrum of metformin biologic actions has been notoriously laborious to establish. After absorption through the high small-intestine mucosa, this drug circulates chiefly unbound to plasma proteins, being concentrated in both enterocytes and hepatocytes, and is excreted by the kidney, without undergoing measurable metabolization[9].

Inhibition of gluconeogenesis by metformin reliably decreases endogenous glucose production[9], and the main mechanism by which this is accomplished has recently been elucidated by a series of studies by *Madijaru et al*. Through an array of experiments undertaken in rats, infusion of metformin to levels equivalent to those in the therapeutic range achieved in type 2 diabetics, it has been established that the non-competitive inhibition of the mitochondrial enzyme glycerophosphate dehydrogenase, which catalyses the conversion of glycerol-3-phosphate to dyhidroxyacetone phosphate (DHAP), constitutes the main mode of action of this drug[1,10]. Notably, the change in cytosolic NADH-NAD ratio secondary to the diminished levels of DHAP down-regulates the gluconeogenic pathway by impairing the conversion of lactate to pyruvate, thus preventing the use of both pyruvate as well as glycerol as gluconeogenic precursors[10], decreasing the generation of glucose, and mediating the subsequent hypoglycemic effect of metformin (Fig. 1).

Figure 1. Metformin mode of action



Metformin inhibits the mitochondrial isoform of the enzyme glycerophosphate dehydrogenase thus altering the NADH-NAD ratio due to decreased levels of DHAP. This impairs the conversion of lactate to pyruvate, blunting gluconeogenesis and therefore hepatic glucose output. mGDP: mitochondrial glycerophosphate dehydrogenase; cGDP: cytoplasmatic glycerophosphate dehydrogenase DHAP: dyhidroxyacetone phosphate; LDH: lactate dehydrogenase.

Despite the elucidation of the aforementioned mechanism of action, metformin has remarkably far more biologic effects. Gene expression modulation has also been postulated to play an important role in the hypoglycemic effect of this drug. *Canton et al* have reported that metformin induced, AMPK-dependent, increase in the levels of SIRT1 and GCN5, key regulators of gluconeogenic pathway gene expression, constitute a potential mechanism of inhibition of hepatic gluconeogenesis by metformin[11]. In addition, AMPK-mediated induction in the expression of the orphan nuclear receptor SHP has also been implicated as a mechanism of inhibition of gluconeogenic gene activity, namely PEPCK and G6Pase down-regulation, secondary to the up-regulation of SHP[12]. Nonetheless, many other proposed mechanism of action abound, consequent of many studies undertaken over the years. These range from delayed intestinal absorption of glucose, interference with glucagon signalling, and increased enterocyte generation of lactate, to increased levels of glucagon-like peptide 1 and inhibition of mitochondrial complex I[1]. This plethora of proposed biologic effects underscores the complexity of the full clinical spectrum of metformin actions, both therapeutic as well as in the context of metformin intoxication.

Lactic acidosis, defined as an arterial pH≤7.35 and a lactate level ≥5 mmol/L[13], secondary to the administration of metformin- the so-called *metformin-associated lactic acidosis* (MALA)- is a notoriously rare occurrence, taking into account the prevalent use of the drug[6,7]. The circumstances under which metformin may induce lactic acidosis are either accumulation of the drug due to a medical condition impairing the ability of excretion, in patients taking therapeutic doses; or the ingestion, accidental or voluntary, of an excessive amount, acutely surpassing the excretory capacity, even though a cut-off value of the necessary quantity to induce toxicity remains elusive[6].

The role played by metformin in the induction of lactic acidosis, and the mechanisms involved in this toxicity haven't been thus far fully clarified. It is important to note that metformin has inherited a class-effect, given that the development of lactic acidosis has been well-established following phenformin administration, another biguanide, through inhibition of oxidative phosphorylation, both peripheral and hepatic, with ensuing potentiation of anaerobic metabolism and lactate generation[3]. At the hepatocyte level, at least, this effect appears to be related to the organic cation transporter 1-mediated uptake of biguanides and intracellular concentration of the drug[14]. Even so, it must be emphasized that the potential of

metformin to induce lactic acidosis is reportedly 140 times less than that of phenformin[14].

Several studies have focused on the inhibitory effect of metformin on the components of the respiratory chain. This effect on oxygen consumption has been observed in patients following biguanide intoxication, in which mitochondrial toxicity impairs tissue oxygen consumption despite adequate O_2 delivery[15]. Such toxicity has also been reported to induce lactate production in peripheral blood monocytes and platelets, via specific inhibition of mitochondrial complex I[16]. This global impairment of oxygen consumption has been further validated by animal model studies, where it was observed that metformin, in addition to induction of lactic acidosis, also caused widespread mitochondrial dysfunction, both at the hepatic and peripheral level[17]. Further substantiation of the respiratory inhibition by metformin ensues from the fact that lactic acid infusion failed to translate into blunting of oxygen consumption at the cellular level[17], revealing a direct effect of the drug, thus exculpating lactate from this responsability. These findings highlight that, in spite of the central part played by the liver in both metformin therapeutic and toxic actions, the peripheral activity of this drug is key to the understanding of the whole clinical picture of metformin intoxication.

Metformin intoxication- Clinical presentation and diagnosis

The clinical picture of acute metformin intoxication presents itself as a diagnostic challenge due to its remarkably uncharacteristic features. First and foremost, the differential diagnosis of lactic acidosis is vast[18], and a myriad of conditions course with its development (Table I)[18]. Therefore, an increased arterial lactate level coupled with reduced pH in a patient under metformin therapy is not necessarily indicative of MALA. Further complicating this diagnosis is the fact that the majority of patients at risk of developing MALA- *i.e.* metformin-treated type 2 diabetics- have an inherently higher probability of lactic acidosis unrelated to drug therapy[3].

In the literature, the most commonly related features of MALA are of gastrointestinal nature, including anorexia, nausea, vomiting, abdominal pain, and diarrhoea[3, 5]. The occurrence of hypothermia, hypotension and respiratory failure in

severe cases have also been reported[5], as well as asymptomatic presentations[6]. Hence, the difficulty of recognition of MALA cannot be overstated, and a considerable index of suspicion and awareness of this entity are fundamental.

In the presence of a patient with suspected MALA, it should be kept in mind that the overwhelming majority of cases occur in individuals with an intrinsically elevated risk of lactic acidosis related to underlying conditions, and that fundamentally all diabetic patients may develop the latter when their circulatory system is put under stress[3]. Also obscuring an eventual diagnosis of MALA is the fact that plasma metformin levels, besides not being readily available in most emergency settings[7,19,20], are surprisingly not as reliable as it would appear. The finding of low plasma concentrations of the drug should be interpreted as a negative predictor of MALA[7], but the opposite, however, might not be applicable. In fact, the relation between serum metformin levels and hiperlactacidemia is far from being linear[7,19], and even concentrations within the normal therapeutic range have been reported[19] in patients with MALA. Nonetheless, MALA should be pondered in the differential diagnosis of any patient presenting with a metabolic acidosis and elevated lactate levels, after systematic exclusion of other precipitating causes[20], and in accordance with the history of metformin dosage and latest administration[7]. It should be kept in mind, however, that the preceding criteria allow only for the establishment of a probable diagnosis of MALA, but even so this should prompt expeditious institution of therapeutic measures in order to establish the adequate management of such patients.

Table I. Causes of lactic acidosis

Shock (cardiogenic or hypovolemic), heart failure, trauma	Metformin
Sepsis	Nucleoside reverse-transcriptase inhibitors
Severe hypoxemia	Cocaine
CO poisoning	Methanol, ethylene glycol, diethylene glycol, propylene glycol
Severe anemia	Salicylates
Strenuous exercise, seizures	Cyanide
Diabetes mellitus	B_2-agonists
Malignancy	Propofol
Liver disease	Thiamine deficiency
Pheochromocytoma	

Management strategies

Treatment of suspected MALA entails intensive care-based management, being the primary objective the restitution of acid-base equilibrium, with emphasis on enhancement of ventilation in order to allow compensation of the acidosis[7]. From this supportive approach stems the importance of addressing any underlying conditions that may hinder patient recovery[2,5,21], as well as maintenance of circulatory integrity with the use of vasopressor medication[22]. In the exceptional instances where the diagnosis of MALA is made following ingestion of a large amount of metformin, activated charcoal might be used in order to prevent further intestinal absorption of the drug[6,8].

Traditionally, renal replacement techniques have been advocated as a vital component of managing such patients, and several studies have reported its usefulness in this setting. Lactate and metformin are both effectively dialyzable[7], but this does not in itself grant haemodialysis its value in the management of MALA. Rather the rapid correction of volume, osmolality, and pH derangements in an acutely ill subject are the main mechanism through which renal substituting interventions exert their beneficial effect[7,13,23]. Considering metformin's tissue accumulation and high volume of distribution[8], and the fact that its elimination occurs in two distinct phases[2], it is deemed as generally more effective to institute prolonged haemodialysis in this setting[2,23]. Regarding modalities of renal replacement therapies, comparison between conventional haemodialysis (HD) and continuous veno-venous haemodialysis (CVVHD) has failed to yield results favouring one or the other[23], being the latter best suited for patients whose baseline cardiovascular status impedes the use of HD[8,23]. Notwithstanding, no clear agreement exists between peers considering the systematic implementation of neither this measure nor the exact parameters that warrant it in the context of MALA. Namely, there is no strict, widely applicable set of rules regarding when to institute dialysis. However, some have been proposed as possible indications, specifically the existence of serious concomitant pathology, the degree of criticality at presentation, a blood pH value lower than 7.1, as well as failure of supportive treatment, advent of renal insufficiency and, finally, the presence of a fluid overload[23].

Prognosis

Predicting the outcome of MALA has traditionally been notoriously difficult. Given that this condition arises from metformin intoxication (either from failure of excretion or from excessive ingestion) originating a lactic acidosis, it would stand to reason that the plasmatic levels of these substances could have significant implications. However, this has not been indeed verified by most studies. Regarding metformin, no clear correlation between its plasmatic levels and either degree of neither lactacidemia nor mortality has been systematically established[2,22,24]. Also noteworthy is the finding that the degree of renal impairment as measured by seric creatinine did neither increase the risk of mortality nor predict the degree of accumulation of both lactate and metformin[24]. The prognostic impact of renal replacement techniques, despite being generally indicated, is yet to be fully evaluated, and no concrete data exist on their effect on survival. In addition, it has been reported equal survival between patients who had undergone dialysis compared to those who had not[25]. Adding to the intricacy of prognostic evaluation, better outcomes have actually been observed in the patients whose blood metformin concentrations were actually higher compared with non-survivors[4].

Documented variables that adversely impact mortality are sepsis, acute cardiovascular occurrences and end-stage liver failure[24], as well as a decreased prothrombin time (PT) upon admission[2]. The latter measurement has shown predictive power for mortality[2] in the absence of underlying chronic hepatic conditions, possibly indicating a direct acute hepatic damage attributable to metformin. PT determination might consequently be a valuable tool when risk stratification is undertaken in MALA patients.

Given the limited number of studies, it is rather difficult to provide well-defined signs for increased risk of unfavourable outcomes. What currently has been established is that prognosis depends mostly on the severity of the underlying conditions[2,4,23]. Also, when comparing MALA ensuing ingestion of an excessive amount of metformin (voluntary or otherwise) with MALA resulting from drug under excretion due to underlying illness, the prognosis of the former appears much more favourable[2,7] if prompt management is commenced. It has also been reported that, taking into account the degree of acidemia, the prognosis of MALA isn´t as bleak as the prognosis of lactic acidosis resulting from other causes[22].

Stemming from the notions above, the management of MALA and improvement of outcomes relies essentially on the recognition of metformin as an aetiological factor, either causative or precipitating; the rapid evaluation of functional status by use of one of several scales, such as the Logistic Organ Dysfunction System (LODS), Simplified Acute Physiology Score II (SAPS II) and Sequential Organ Failure Assessment (SOFA); and finally, the hasty institution of supportive care and specific therapy.

Conclusions

The cumulative understanding of the full spectrum of metformin biologic actions and the increasing awareness of MALA merit further studies in order to fully elucidate the true role played by this drug. The difficulty of establishing clear protocols regarding the management of MALA is partially explained by the scarcity of prospective works in which careful patient selection and focused laboratory tests- mainly metformin plasma level determination- have taken place. Thus, an effort should be made in designing and implementing studies in order to fully characterize this condition and accompanying clinical picture.

Throughout literature, the severity of MALA has largely been overstated, perhaps due to the history of severe lactic acidosis induced by phenformin, another biguanide. However, regardless of the rarity of severe metformin intoxication, it assumes great importance due to the vast number of patients potentially at risk, most of whom are afflicted by serious comorbid conditions. This is underscored also by the fact that prognosis is largely influenced by the patient's health status and, unlike true over dosage in an otherwise healthy individual, MALA in the setting of underlying pathology adds to a much poorer outcome. Given that true parameters of achieving a diagnosis with certainty are lacking, hasty institution of supportive care and renal replacement techniques appears adequate in the management of this condition, and doing so may be key to improving individual outcomes.

Keeping this in mind, it is crucial to state that, despite the clinical implications of MALA, metformin is a drug with more than proven effectiveness, and its benefits are not limited to its hypoglycemic control in diabetics, exerting multiple therapeutic actions in various organ systems. It has been for years the foundation of treatment of type 2 Diabetes, and the experience with its use coupled with metformin's safety

profile in most situations, as well as an ever-expanding knowledge of its actions, mean that it most likely should remain so.

Disclosure

The authors have no financial conflicts of interest to declare.

Bibliography

1. Phimister E, Ferrannini E. The Target of Metformin in Type 2 Diabetes. New England Journal of Medicine. 2014;371(16):1547-1548.
2. Seidowsky A, Nseir S, Houdret N, Fourrier F. Metformin-associated lactic acidosis: A prognostic and therapeutic study*. Critical Care Medicine. 2009;37(7):2191-2196.
3. Heaf J. Metformin in Chronic Kidney Disease: Time for a Rethink. Peritoneal Dialysis International. 2014;34(4):353-357.
4. van Berlo-van de Laar I, Vermeij C, Doorenbos C. Metformin associated lactic acidosis: incidence and clinical correlation with metformin serum concentration measurements. Journal of Clinical Pharmacy and Therapeutics. 2010;36(3):376-382.
5. Prikis M, Mesler E, Hood V, Weise W. When a friend can become an enemy! Recognition and management of metformin-associated lactic acidosis. Kidney International. 2007;72(9):1157-1160.
6. Kopec K, Kowalski M. Metformin-Associated Lactic Acidosis (MALA): Case Files of the Einstein Medical Center Medical Toxicology Fellowship. Journal of Medical Toxicology. 2012;9(1):61-66.
7. Lalau J. Lactic Acidosis Induced by Metformin. Drug Safety. 2010;33(9):727-740.
8. Rifkin S, McFarren C, Juvvadi R, Weinstein S. Prolonged Hemodialysis for Severe Metformin Intoxication. Renal Failure. 2011;33(4):459-461.
9. Wood A, Bailey C, Turner R. Metformin. New England Journal of Medicine. 1996;334(9):574-579.
10. Madiraju A, Erion D, Rahimi Y, Zhang X, Braddock D, Albright R et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;510(7506):542-546.

11. Caton P, Nayuni N, Kieswich J, Khan N, Yaqoob M, Corder R. Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. Journal of Endocrinology. 2010;205(1):97-106.
12. Kim Y, Park K, Lee Y, Park Y, Kim D, Nedumaran B et al. Metformin Inhibits Hepatic Gluconeogenesis Through AMP-Activated Protein Kinase-Dependent Regulation of the Orphan Nuclear Receptor SHP. Diabetes. 2007;57(2):306-314.
13. Lalau J, Race J. Lactic Acidosis in Metformin-Treated Patients. Drug Safety. 1999;20(4):377-384.
14. Wang D. Involvement of Organic Cation Transporter 1 in the Lactic Acidosis Caused by Metformin. Molecular Pharmacology. 2003;63(4):844-848.
15. Protti A, Russo R, Tagliabue P, Vecchio S, Singer M, Rudiger A et al. Oxygen consumption is depressed in patients with lactic acidosis due to biguanide intoxication. Critical Care. 2010;14(1):R22.
16. Piel S, Ehinger J, Elmér E, Hansson M. Metformin induces lactate production in peripheral blood mononuclear cells and platelets through specific mitochondrial complex I inhibition. Acta Physiologica. 2014;213(1):171-180.
17. Protti A, Fortunato F, Monti M, Vecchio S, Gatti S, Comi G et al. Metformin overdose, but not lactic acidosis per se, inhibits oxygen consumption in pigs. Critical Care. 2012;16(3):R75.
18. Ingelfinger J, Kraut J, Madias N. Lactic Acidosis. New England Journal of Medicine. 2014;371(24):2309-2319.
19. Lalau J, Race J. Metformin and lactic acidosis in diabetic humans. Diabetes Obes Metab. 2000;2(3):131-137.
20. Dell'Aglio D, Perino L, Kazzi Z, Abramson J, Schwartz M, Morgan B. Acute Metformin Overdose: Examining Serum pH, Lactate Level, and Metformin Concentrations in Survivors Versus Nonsurvivors: A Systematic Review of the Literature. Annals of Emergency Medicine. 2009;54(6):818-823.
21. Gan S. Biguanide-associated lactic acidosis. Case report and review of the literature. Archives of Internal Medicine. 1992;152(11):2333-2336.
22. Friesecke S, Abel P, Roser M, Felix S, Runge S. Outcome of severe lactic acidosis associated with metformin accumulation. Critical Care. 2010;14(6):R226.

23. Nguyen H, Concepcion L. Metformin intoxication requiring dialysis. Hemodialysis International. 2011;15:S68-S71.
24. Stades A, Heikens J, Erkelens D, Holleman F, Hoekstra J. Metformin and lactic acidosis: cause or coincidence? A review of case reports. J Intern Med. 2004;255(2):179-187.
25. Peters N, Jay N, Barraud D, Cravoisy A, Nace L, Bollaert P et al. Metformin-associated lactic acidosis in an intensive care unit. Critical Care. 2008;12(6):R149.

Agradecimentos

Em primeiro lugar, gostaria de agradecer à Doutora Carla Araújo todo o apoio, paciência e dedicação ao longo da realização deste trabalho. Foi de facto um privilégio.

Naturalmente, agradeço a toda a minha família. Aos meus Avós, pelo apoio e orgulho incondicionais, pela abnegação que sempre demonstraram, e pelo exemplo que estabeleceram. Aos meus Pais, cujo amor e entrega, em qualquer circunstância demonstraram. Toda a sua vida vivida em função dos filhos, um espírito de sacrifício inabalável, agradeço acima de tudo a educação que me proporcionaram. À minha irmã, melhor amiga, porto seguro, dedico também o meu trabalho e, acima de tudo, o meu orgulho. A meus Tios, que elogio maior lhes posso fazer que o de dizer que sempre me senti amado como um filho.

Os velhos amigos, companheiros há anos que parecem infindáveis, agradeço principalmente todas as palavras de apoio, toda a coragem que demonstraram quando a mim ma faltou. Obrigado Pedro Reis, Filipe Borda d'Água, Rui Especial, Luís Veludo, Pedro Pinto e João Silva. Se hoje sou alguém, sou-o também graças a vós. Os anos continuarão a passar, indubitavelmente, mas sempre com a vossa companhia, lealdade e dedicação.

Aos companheiros desta jornada que está prestes a terminar, aqueles que passaram comigo as imensas tormentas e alegrias deste Curso, um enorme obrigado. Maior orgulho que, por daqui a meses, vos chamar "colegas", é o de vos chamar Amigos, agora e sempre. Sérgio Sousa, Diogo Pereira, Manuel Vilela, Nuno Jesus e Mariana Liz, foi uma honra, nunca esquecerei os tempos de Faculdade, nunca esquecerei que me tornei Médico ao vosso lado.

Nas vésperas de realizar o meu sonho, constato que já sou feliz.

A todos vós, um enorme e sentido obrigado.

Anexos

Nephrology & Hypertension
ISSN 2183-1289
The journal is indexed in Thompson Reuter's SciELO Citation Index

INSTRUCTIONS FOR AUTHORS

Manuscripts must be submitted online http://rpnh.spnefro.pt. Once you have prepared your manuscript according to the Instructions below, please pay particular attention to the sections on Informed Consent and Ethics and Disclosure.

The text should be double-spaced. The corresponding author should describe the contributions of all authors to the article. Manuscripts should bear the name, address and e-mail of the corresponding author.

Should the manuscript be accepted for publication the authors will be asked to give signed consent for publication in a letter which must contain the statement that "the results presented in this paper have not been published previously, in whole or in part, except in abstract form".

Title Page: The title page should carry the full title of the paper and the first name, middle initial (if applicable) and last name of each author, plus the names and addresses of the respective institutions where the work was done; in the case of different institutions the author(s) should be identified using superscript Arabic numerals. In the case of Portuguese language authors, the title must also be translated into Portuguese.

Abstract: Not more than 300 words. Abbreviations should not be used.
In the case of Portuguese language authors, the abstract must also be translated into Portuguese.

Key-Words: Not more than 6, in alphabetical order, and the terms used (when possible) should be from the Medical Subject Headings list of the Index Medicus. In the case of Portuguese language authors, the key-words must also be translated into Portuguese.

References: Authors are responsible for bibliographic accuracy. All the references, including those with only electronic sources, should be cited according to the "Vancouver Citation Style" which can be consulted on the Internet at: http://library.vcc.ca/downloads/VCC_VancouverStyleGuide.pdf

References must be numbered consecutively in the order in which they are cited in the text. Each reference should give the name and initials of all authors unless they are more than six, when only the first three should be given followed by et al. Authors' names should be followed by the title of the article, journal abbreviations according to the style used in Index Medicus, the year of publication, the volume number and the

first and last page numbers. For papers in the course of publication, “in press” replaces the date; the journal name must be given in the references. Manuscripts that are unpublished, in preparation, or submitted, and personal communications should not be cited in the reference list but may appear parenthetically in the text. References to books should contain the author(s) name(s) and initials, the title of the book, followed by place of publication, publisher, year, and relevant pages. Websites must be referenced by the following order: title, URL and access date.

Examples

1. *Journals*:
Hogan J, Mohan P, Appel GB. Diagnostic tests and treatment options in glomerular disease: 2014 update. Am J Kidney
Dis 2014;63(4):656-666

2. *Books*:
Morris Peter, Knechtle Stuart. Kidney Transplantation - Principles and Practice. 7th Edition. Saunders, 2014:72

3. *Website*:
Substitutive Renal Therapy of Chronic Renal Disease in Portugal.
Available at http:www.spnefro.pt/comissoes_Gabinete_registo_2013/registo_2013. Accessed October 6, 2013.

4. *Published Meeting Abstract*:
Jorge Silva, Jorge Antunes, Telmo Carvalho, Pedro Ponce.
Efficacy of preventing hemodialysis catheter infections with citrate lock (Encontro Renal abstract SE001). Port J Nephrol Hypert 2011; 25(1):56

Tables: Tables should supplement, not duplicate, the information in the main text. References to tables should be made in order of appearance in the text and should be in Roman numerals in brackets, e.g. (Table II). Each table should be typed on a separate sheet and have a brief heading describing its contents.

Figures: All illustrations (transparencies, photographs, diagrams, graphs, etc.) should be labelled consecutively in Arabic numerals (Fig. 1, 2...), according to their relative positions in the text. If a figure has been published before, the original source must be acknowledged and written permission from the copyright holder must be submitted with the material.

Informed Consent and Ethics: Identifying details of patients should not be published in descriptions unless the information is essential for scientific purposes and the patient gives written informed consent for publication. Patients shown in

photographs should have their identity obscured or the picture must be accompanied by written permission to use the photograph.

When reporting experiments on human subjects, it is mandatory to indicate whether the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975 (revised in 2008) and, in the case of renal transplant, the Declaration of Istanbul.

When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Disclosure: Each manuscript must include a conflict of interest statement before the References section. The disclosure statement will describe the sources of any support for the work in the form of grants, consulting fees or honoraria from industry, equipment, provision of drugs, travel related with the study or any combination thereof. Any relevant financial activities outside the submitted paper but considered stakeholders in the field must be detailed. The corresponding author should provide a Conflict of Interest Declaration describing the possible financial interests of all the authors. The absence of any interest must also be declared.

Acknowledgements should be located in the manuscript body before the conflict of interest statement.